



CO₂ elevation modulates the response of leaf gas exchange to progressive soil drying in tomato plants

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ABSTRACT

The objective of this study was to investigate the response of leaf gas exchange of tomato plant to progressive drought stress under ambient ($a[\text{CO}_2]$, 400 ppm) and elevated ($e[\text{CO}_2]$, 800 ppm) atmospheric CO₂ concentration. The fraction of transpirable soil water (FTSW) was used to evaluate soil water status in the pots. The results showed that stomatal conductance (g_s) and transpiration rate (T_r) were significantly lower while the net photosynthetic rate (A_n) was significantly higher in plants grown under $e[\text{CO}_2]$ than those under $a[\text{CO}_2]$ at onset of drought stress. Along with soil drying, the FTSW thresholds at which g_s and A_n started to decrease were significantly lower in plants grown under $e[\text{CO}_2]$ as compared to plants grown under $a[\text{CO}_2]$. The intrinsic water use efficiency and instantaneous water use efficiency of plants grown under $e[\text{CO}_2]$ was significantly higher than those under $a[\text{CO}_2]$. Under $e[\text{CO}_2]$, the drought-stressed plants had greater leaf area, dry matter and water use efficiency than those grown under $a[\text{CO}_2]$. $e[\text{CO}_2]$ notably enhanced shoot C concentration while decreased shoot N concentration hereby increased the C:N ratio. With the decrease of FTSW, the concentration of abscisic acid in leaf ($[\text{ABA}]_{\text{leaf}}$) and xylem sap ($[\text{ABA}]_{\text{xylem}}$) increased exponentially. When $\text{FTSW} > 0.2$, under both CO₂ environments, g_s decreased linearly with increasing $[\text{ABA}]_{\text{leaf}}$ and $[\text{ABA}]_{\text{xylem}}$; and similar slopes but different intercepts were noticed for the regression lines, indicating that the responsiveness of g_s to ABA was unaffected by CO₂. In conclusion, CO₂ elevation retarded the response of leaf gas exchange to progressive soil drying in tomato plants. This result provides novel knowledge for more precise prediction of plant response to drought stress in a future CO₂-enriched environment.

1. Introduction

Currently, the atmospheric CO₂ concentration ($[\text{CO}_2]$) has exceeded 400 ppm (Tans and Keeling, 2018) and is expected to double at the end of this century (Intergovernmental Panel on Climate Change, 2013), mainly due to anthropogenic activities as fossil fuel burning and deforestation (Qaderi and Reid, 2009). The elevated CO₂ ($e[\text{CO}_2]$) is a potential driver for increasing global temperature and changing precipitation patterns, leading to drought at both regional and global level. Drought is one of the most important factors restricting crop production in many areas of the world (Boyer et al., 2013). Therefore, a better understanding of plant responses to rising CO₂ and drought is essential for enhancing crop yield and water use efficiency (WUE) in future climate.

Stomata are tiny openings in plant tissue that allow for gas exchange between plants and atmosphere. Stomatal aperture is regulated by various environmental factors, including atmospheric CO₂ concentration (Mansfield et al., 1990; Assmann, 1999; Azoulay-Shemer et al., 2015), leaf to air vapour pressure deficit (VPD) (Yong et al., 1997; Bunce, 1998; Li and Li, 2014), and water status of the plant (Liu et al., 2005). In relation to plants grown at ambient CO₂ concentration, stomata tended to open at low CO₂ level but tended to close at $e[\text{CO}_2]$ (Lee et al., 2008; Merilo et al., 2014). During progressive soil drying, plants can sense the water availability around the roots and respond by sending chemical signals (mainly xylem-borne abscisic acid, $[\text{ABA}]_{\text{xylem}}$) to the shoot, narrowing stomatal apertures, thus decrease stomatal conductance (g_s) (Zhang and Davies, 1990; Liu et al., 2005). Nevertheless, recent study by Zhang et al. (2018) showed that rapid

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ABA biosynthesis predominantly occurs in the leaves, and not in other tissues in angiosperms following a short-term reduction in cell volume generated by the application of external pressure. It is widely accepted that, both $e[\text{CO}_2]$ and soil water deficit could reduce g_s (Xu et al., 2016; Yan et al., 2017), and soil water deficit has a stronger influence on g_s than $e[\text{CO}_2]$ when combined factors exposed (Leakey et al., 2006). Some researchers suggested that $e[\text{CO}_2]$ could relieve drought damage by reducing g_s and transpiration rate (T_r) to sustain a high leaf water potential (Tardieu and Simonneau, 1998; Tausz-Posch et al., 2015). However, recent study revealed that g_s of plant leaves under $e[\text{CO}_2]$ decreased later but more sharply than that under $a[\text{CO}_2]$ during progressive soil drying (Yan et al., 2017). Moreover, Yan et al. (2017) found that at $a[\text{CO}_2]$ the decrease of g_s in tomato leaves was mainly regulated by the $[\text{ABA}]_{\text{xylem}}$ at moderate drought stress; while under $e[\text{CO}_2]$ the g_s was predominately controlled by leaf turgor pressure. While, there was no information related to leaf ABA ($[\text{ABA}]_{\text{leaf}}$), which may also involve in regulating g_s of tomato plants subjected to drought in different CO_2 environments. In addition, whether $e[\text{CO}_2]$ could modulate the response of g_s to water deficits remains largely elusive, and the mechanisms by which g_s is regulated under combined drought and $e[\text{CO}_2]$ are still need to be investigated.

It is well recognized that plants grown at $e[\text{CO}_2]$ had increased leaf photosynthetic rate (A_n), decreased g_s and T_r , thus resulting in improved WUE at stomatal and leaf scales (Drake et al., 1997; Kang et al., 2002; Yan et al., 2017; Wei et al., 2018). Furthermore, the increased A_n lowered T_r of plants grown under $e[\text{CO}_2]$ often associated with greater biomass and reduced water consumption resulting in an improved WUE at plant level (Pazzagli et al., 2016). On the other hand, plant N could regulate C metabolism in plants, as N is an essential component for all of the enzymes involving in carbohydrate metabolism, transport and utilization (Huppe and Turpin, 1994). Moreover, mineral concentrations especially N concentration ($[\text{N}]$) in plants grown at $e[\text{CO}_2]$ was often found to decrease, mostly due to the dilution effect by the increased biomass and reduced root N uptake resulting from limited transpiration mass flow of plant (Taub and Wang, 2008; Myers et al., 2014). It is worth noting that at a reduced N nutrition, $e[\text{CO}_2]$ increased cytokinins delivery to the leaf causing stomatal opening (Yong et al., 2000; Teng et al., 2006), which may offset the effect of ABA-induced stomatal closure under moderate drought. It has been reported that carbon and nitrogen ratio ($[\text{C:N}]$) in plant dry biomass could indicate the amount of carbon fixed per unit N acquired, and could be used as an indicator of nitrogen use efficiency (NUE) (Livingston et al., 1999; Wang et al., 2010; Wei et al., 2018). An increased NUE has frequently been observed from plants grown at $e[\text{CO}_2]$ (Reddy et al., 2010). However, the mechanism by which NUE is modified remains unknown when plants exposed to combined drought stress and $e[\text{CO}_2]$ environment.

Therefore, the objective of the present study was to investigate the effects of $e[\text{CO}_2]$ as compared with $a[\text{CO}_2]$ on the response of leaf gas exchange, plant water relations and plant growth in tomato plants to progressive soil drying. Tomato plants were transplanted in two atmospheric $[\text{CO}_2]$ (400 and 800 ppm) environments at 4-leaf stage. Eleven days after transplanting in each cell, 4 plants were well watered and others (20 plants) were exposed to progressive drought stress by withholding irrigation lasting 8 days. Leaf gas exchange, plant water relations, shoot dry mass, WUE in stomatal, leaf and plant scopes, leaf area, concentrations of $[\text{C}]$ and $[\text{N}]$ in leave and stem, and ABA concentration in leaf and xylem sap were measured. It was hypothesized that: 1) $[\text{ABA}]_{\text{leaf}}$ and $[\text{ABA}]_{\text{xylem}}$ would be involved in regulation of g_s during drought stress in both $a[\text{CO}_2]$ and $e[\text{CO}_2]$ environments; and 2) due to the initial lower g_s of plants grown under $e[\text{CO}_2]$, it would retard the decline of g_s during the soil drying.

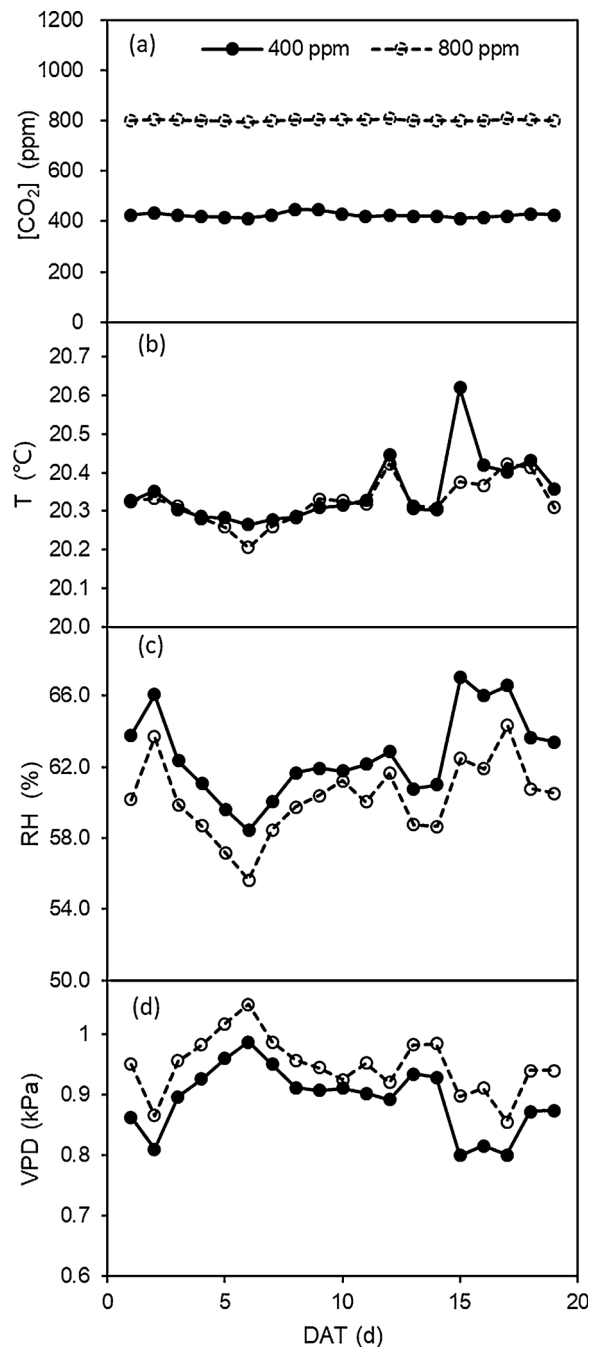


Fig. 1. The average of $[\text{CO}_2]$ concentration, temperature (T), relative humidity (RH) and vapor pressure deficiency (VPD) in greenhouse cells designated at 400 and 800 ppm during the experimental period.

2. Materials and methods

2.1. Experimental setup

Pot experiment was conducted in climate-controlled greenhouses at Faculty of Science, University of Copenhagen, Taastrup, Denmark. Tomato seeds were sown on 14th December 2017. At the 4-leaf stage, seedlings were transplanted to 3 l pots filled with 0.9 kg peat (Pindstrup substrate no. 2, Pindstrup Mosebrug A/S, Pindstrup, Denmark) in greenhouse cells with CO_2 concentration of 400 ppm (ambient CO_2 , $a[\text{CO}_2]$) and 800 ppm (elevated CO_2 , $e[\text{CO}_2]$). The $[\text{CO}_2]$ was sustained by pure CO_2 emission from a bottled tank, released in one point and distributed evenly in the cells through internal ventilation. The $[\text{CO}_2]$ in

the cells was monitored every 6 s by a CO₂ Transmitter Series GMT220 (Vaisala Group, Helsinki, Finland). The climate conditions in the two glasshouse cells were set at: 25/16 ± 2 °C day/night air temperature, 60 ± 2% relative humidity, 16 h photoperiod and > 500 μmol m⁻²s⁻¹ photosynthetic active radiation (PAR) supplied by sunlight plus LDE lamps. The average of daily CO₂ concentration ([CO₂]), temperature (T), relative humidity (RH) and vapour pressure deficiency (VPD) in the cells during the experiment are shown in Fig. 1.

After transplanting, 1.5 cm quartz gravel was covered on the soil surface to minimize evaporation, and pots were watered to saturate condition with a constant weight (i.e., 2.1 kg) until 21st January 2018. From 22nd January, in each cell, four tomato plants were well watered as control, the others (20 plants) were subjected to progressive soil drying by withholding irrigation from the pots for 8 days until the *g_s* decreased to ca. 10% of the control (i.e., when pot weight ca. 0.8 kg). During the progressive soil drying, the plants were harvested five times at different soil water deficits; and at each harvest, 3–4 drought-stressed plants were harvested. Soil water status in the pots were expressed as the fraction of transpirable soil water (FTSW). Total transpirable soil water (TTSW) was the difference between pot weights at 100% pot holding capacity (i.e., 2.1 kg) and when the *g_s* of the drought-stressed plants decreased to ca. 10% of the well-watered control (i.e., 0.8 kg). The daily value of FTSW was estimated as the ratio between the amount of transpirable soil water still remaining in the pot and TTSW:

$$\text{FTSW} = (\text{WT}_n - \text{WT}_t) / \text{TTSW}$$

where WT_n is the pot weight on a given date, and WT_t is the pot weight at the time when *g_s* of stress plants was 10% of the control plants (i.e., 0.8 kg).

Changes of FTSW during the experimental period in the two cells are presented in Fig. 2.

2.2. Measurements

2.2.1. Leaf gas exchange

During the progressive soil drying, leaf gas exchange rates, including net photosynthetic rate (*A_n*, μmol m⁻²s⁻¹), stomatal conductance (*g_s*, mol m⁻²s⁻¹) and transpiration rate (*T_r*, mmol m⁻²s⁻¹) were measured on upper canopy fully expanded leaves between 9:00 to 11:00 h with a portable photosynthetic system (LiCor-6400XT, LI-Cor, NE, USA). Measurements were performed on one leaf per plant at 25°C chamber temperature and 1200 μmol m⁻²s⁻¹ photon flux density, and at *a*[CO₂] of 400 ppm for *a*[CO₂] and 800 ppm for *e*[CO₂] treatment, respectively. Intrinsic water use efficiency (WUE_i, μmol CO₂ mol⁻¹

H₂O) and instantaneous water use efficiency (WUE_{leaf}) were calculated as the ratio of *A_n* to *g_s*, *A_n* to *T_r*, respectively.

2.2.2. Plant water relation

Plant water relation characteristics were measured at the final harvest.

Relative water content (RWC) was determined as:

$$\text{RWC} = (\text{W}_f - \text{W}_d) / (\text{W}_t - \text{W}_d)$$

where *W_f* was the fresh weight of the leaf, *W_t* was the turgid weight of the leaf determined after immersing in distilled water for 2 h, and *W_d* was the dry weight of the leaf determined after 48 h drying in an oven at 75°C.

Leaf water potential (*ψ_l*) was determined with a pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA) on the same leaf for measuring gas exchange. After measuring *ψ_l*, the leaf was divided into two parts, immediately packed in aluminum foils, and frozen in liquid nitrogen and stored at -80°C for measurement of osmotic potential (*ψ_π*) and leaf abscisic acid concentration ([ABA]_{leaf}). *ψ_π* was measured after thawing the leaf sample at 20 °C by using a psychrometer (C-52 sample chambers, Wescor Inc., Logan, UT, USA) connected to a microvoltmeter (HR-33 T, Wescor, Logan, UT, USA). Turgor press (*ψ_p*) was the difference between *ψ_l* and *ψ_π*.

2.2.3. Water consumption, dry matter, water use efficiency and leaf area

Water consumption (WU, cm³) was evaluated since the beginning of treatment (the first harvest). Dry mass increase (ΔDM, g) was the difference of dry mass between the first and the final harvest. Water use efficiency (WUE, kg m⁻³) of plant was determined as ΔDM/WU.

Plant leaf area (LA, cm²) was measured with a leaf area meter (LICOR 3100) and specific leaf area (SLA, cm² g⁻¹) was calculated as the ratio of LA to leaf dry mass.

2.2.4. C and N concentration in leaf and stem

The concentration of carbon ([C], g kg⁻¹) and nitrogen ([N], g kg⁻¹) in leaf and stem was determined in samples from the final harvest. Dry samples were thoroughly grounded into powder and analyzed for [C] and [N] using a CHNS/O Elemental Analyzer (Flash 2000, Thermo Fisher Scientific, Cambridge, UK). [C] in shoot was calculated as:

$$[\text{C}]_{\text{shoot}} = ([\text{C}]_{\text{leaf}} \times \text{DM}_{\text{leaf}} + [\text{C}]_{\text{stem}} \times \text{DM}_{\text{stem}}) / (\text{DM}_{\text{leaf}} + \text{DM}_{\text{stem}})$$

the similar way was applied to determine [N]_{shoot} as:

$$[\text{N}]_{\text{shoot}} = ([\text{N}]_{\text{leaf}} \times \text{DM}_{\text{leaf}} + [\text{N}]_{\text{stem}} \times \text{DM}_{\text{stem}}) / (\text{DM}_{\text{leaf}} + \text{DM}_{\text{stem}})$$

[C:N]_{shoot} was the ratio of [C] to [N] in shoot.

2.2.5. Leaf ABA and xylem ABA concentrations

Leaf sample was grounded into powder with liquid nitrogen, and 27–33 mg was weighted and added into a 1.5 ml Eppendorf tube. The ABA was extracted with 1.0 ml milli-Q water on a shaker at 4 °C overnight. The extracts were centrifuged at 14,000 g and 0.7 ml supernatants were collected for [ABA]_{leaf} analysis. At each harvest, xylem sap was collected by pressurizing the potted plant in a Scholander-type pressure chamber following the procedure described by Liu et al. (2006). Briefly, the stem was cut at 5–10 cm above the soil surface of soil and the pot was sealed into the chamber with the cut outside. Pressure was applied gradually to a value equals *ψ_l* of the plant, after discarding the first few droplets of the xylem sap by a piece of tissue paper to avoid contamination (Schurr, 1998), the sap was collected into light-tight Eppendorf tubes and were immediately frozen in the liquid nitrogen and stored at -80 °C for ABA analysis. [ABA]_{leaf} and xylem sap ABA concentration ([ABA]_{xylem}) were assayed by ELISA (Enzyme Linked Immuno Sorbent Assay) following the protocol described by Asch (2000). Before the measurements, a cross-reaction test was

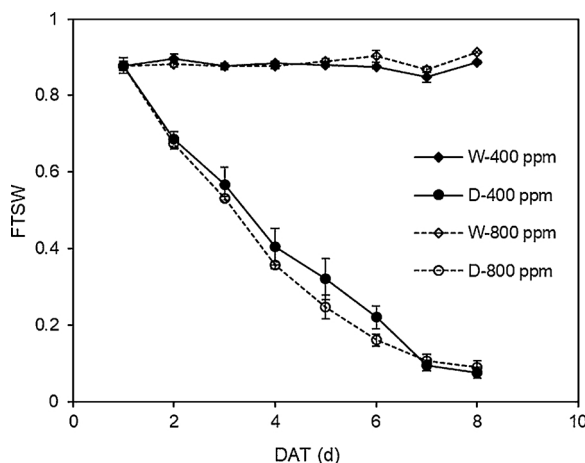


Fig. 2. Change in fraction of transpiration soil water (FTSW) over time (DAT, day after treatment) in well-watered (W) and droughted (D) pots under *a*[CO₂] and *e*[CO₂]. Error bars indicate the standard error of the means (S.E.) (N = 3–4).

performed on the leaf samples indicating no cross reaction was detected.

2.3. Data analysis and statistics

The responses of stomatal conductance (g_s), net photosynthetic rate (A_n), transpiration rate (T_r), intrinsic water use efficiency (WUE_i , A_n/g_s) and instantaneous water use efficiency (WUE_{leaf} , A_n/T_r) were described by a linear-plateau model:

If $FTSW > C$, $y = y_{initial}$;

If $FTSW < C$, $y = y_{initial} + a \times (FTSW - C)$

where y means g_s , A_n , T_r , WUE_i or WUE_{leaf} , $y_{initial}$ means $g_{s\ max}$, $A_{n\ max}$, $T_{r\ max}$, $A_{n\ max}/g_{s\ max}$ or $A_{n\ max}/T_{r\ max}$, respectively; a was the slope of the linear equation, C was the FTSW threshold at which y started to diverge from $y_{initial}$. The parameters y , a and C were estimated by PROC NLIN of PC SAS 9.4 (SAS Institute Inc., Cary, NC, USA, 2002–2012).

Data were subject to two-way Analysis of Variance (ANOVA) to analyze the effects of water and CO_2 treatment on RWC, ψ_l , ψ_p , ψ_p , WU, ΔDM , WUE, LA, SLA, $[C]_{shoot}$, $[N]_{shoot}$ and $[C:N]_{shoot}$, and data were compared using Duncan Test in SPSS 21.

3. Results

3.1. Leaf gas exchange

At onset of drought stress, plants grown under $e[CO_2]$ had notably 34.8% lower stomatal conductance (g_s) than those grown under $a[CO_2]$ (Fig. 3a; Table 1). Along with the progressive soil drying, g_s of the plants grown under $e[CO_2]$ began to decline linearly when FTSW decreased to 0.40, which was significantly lower than those grown under $a[CO_2]$, i.e., 0.61 (Fig. 3a; Table 1). Upon onset of drought stress, the net photosynthetic rate (A_n) in plants grown under $e[CO_2]$ was 24.5% higher than those under $a[CO_2]$, and it started to decrease at lower FTSW threshold (i.e., 0.24) than did the plants grown under $a[CO_2]$ (i.e., 0.36) (Fig. 3b; Table 1). Compared to the plants grown under $a[CO_2]$, transpiration rate (T_r) of plants grown under $e[CO_2]$ was 31.9% lower though the FTSW thresholds at which T_r started to decrease were similar in plants grown under both CO_2 environments (Fig. 3c; Table 1).

After imposition of drought stress, the initial intrinsic water use efficiency (WUE_i , A_n/g_s) of plants grown under $e[CO_2]$ was ca. 1.5 times higher than those grown under $a[CO_2]$ (Fig. 4a; Table 1). Similarly, the instantaneous water use efficiency (WUE_{leaf} , A_n/T_r) was improved by 96.1% by grown under $e[CO_2]$ (Fig. 4b; Table 1). The WUE_i and WUE_{leaf} was kept constant at the initial value until FTSW decreased to ca. 0.30–0.40 and increased linearly as FTSW decreased further (Fig. 4). No difference was found in the FTSW thresholds at which the WUEs started to increase (Table 1).

3.2. Plant water relations

In the final harvest, relative water content (RWC), leaf water potential (ψ_l) and osmotic potential (ψ_π) were significantly affected by water treatment (Table 2), and turgor pressure (ψ_p) was affected by neither CO_2 nor water treatment (Table 2). In relation to the well-watered plants, RWC, ψ_l and ψ_π in the drought-stressed plants were significantly lower than the well-watered controls, while CO_2 treatment had no significant effect on those variables (Table 2).

3.3. Water consumption, dry matter accumulation and plant water use efficiency

During the irrigation treatment (from the 1st to the last harvest, 8 days in total), water consumption (WU) was solely significantly affected by water treatment (Table 2). Regardless of CO_2 growth environments,

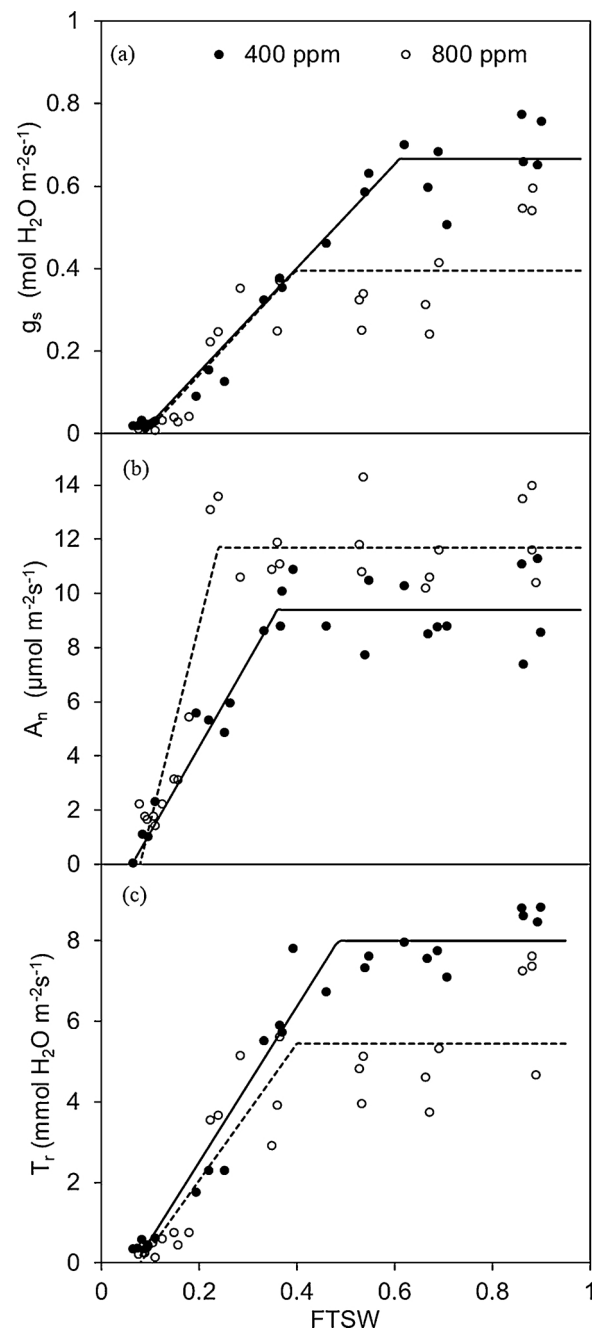


Fig. 3. Changes of stomatal conductance (g_s), net photosynthetic rate (A_n) and transpiration rate (T_r) of tomato leaves grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying.

WU in plants under drought stress was reduced by 35.43% compared with well-watered plants (Table 2). Dry matter increase (ΔDM) and plant water use efficiency (WUE) were significantly affected by both CO_2 and water treatments (Table 2). Water-stressed plants had lower ΔDM and WUE (averagely decreased by 46.1% and 16.3%, respectively) than those under well-watered condition across the CO_2 environments (Table 2). Additionally, for irrigated and stressed plants grown under $e[CO_2]$, ΔDM was improved by 14.7% and 28.3% than those grown under $a[CO_2]$ (Table 2). The well-watered and the drought-stressed plants grown under $e[CO_2]$ had 18.3% and 34.2% higher WUE than those grown under $a[CO_2]$, respectively (Table 2).

Table 1

Significant test for linear-plateau model parameters of stomatal conductance (g_s), net photosynthetic rate (A_n), transpiration rate (T_r), intrinsic water use efficiency (WUE_i , A_n/g_s) and instantaneous water use efficiency (WUE_{leaf} , A_n/T_r). The data is presented in Figs. 3 and 4.

	g_s			A_n			T_r			WUE_i			WUE_{leaf}		
	A	C	$g_{s\ max}$	a	C	$A_{n\ max}$	a	C	$T_{r\ max}$	a	C	$A_{n\ max}/g_{s\ max}$	a	C	$A_{n\ max}/T_{r\ max}$
400 ppm	1.26	0.61	0.67	31.58	0.36	9.40	19.34	0.48	8.01	−111.00	0.44	14.75	−4.44	0.44	1.18
800 ppm	1.28	0.40	0.40	72.93	0.24	11.70	16.99	0.40	5.46	−462.60	0.28	36.35	−16.10	0.35	2.31
Sig.	0.93	0.01	< 0.001	< 0.001	< 0.001	< 0.001	0.49	0.11	< 0.001	0.06	0.08	0.02	0.07	0.43	0.04

a, the slope of the linear part; C, the threshold at which the parameters start to decrease due to drought stress; $g_{s\ max}$, $A_{n\ max}$, $T_{r\ max}$, $A_{n\ max}/g_{s\ max}$ and $A_{n\ max}/T_{r\ max}$, the initial values of the variables when the plants were not significantly affected by drought.

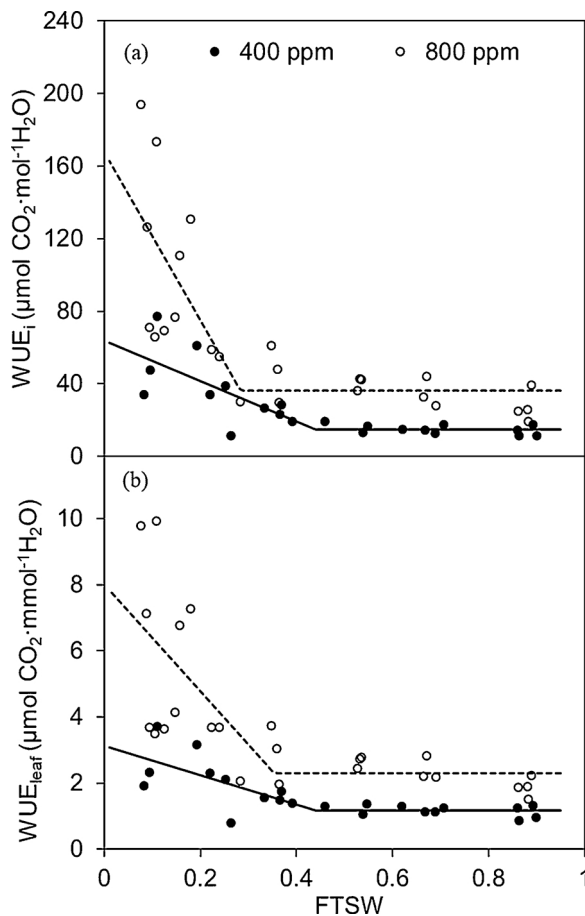


Fig. 4. Changes of intrinsic water use efficiency (WUE_i , A_n/g_s) and leaf water use efficiency (WUE_{leaf} , A_n/T_r) of tomato leave under $a[CO_2]$ and $e[CO_2]$ during the progressive soil drying.

3.4. Leaf area and specific leaf area

Leaf area (LA) of the plants was significantly affected by CO_2 , water and $CO_2 \times$ water interaction (Table 2). Although under well-watered condition the LA was identical between plants grown at the two CO_2 growth environments, under drought stress plants grown at $e[CO_2]$ had a 16.1% greater LA than those grown at $a[CO_2]$. LA was 37.0% lower in the drought-stressed plants relative to the well-watered plants (Table 2). Specific leaf area (SLA) was significantly affected by the CO_2 and water treatment (Table 2). SLA in plants grown under $e[CO_2]$ was 9.8% lower compared with that grown under $a[CO_2]$. Under $a[CO_2]$, drought-stress plants had 4.8% lower SLA than the well-watered plants (Table 2).

3.5. Concentration of C and N in shoot

Shoot C concentration ($[C]_{shoot}$) was significantly affected by CO_2 and $CO_2 \times$ water interaction (Fig. 5a). Plants grown at $e[CO_2]$ had greater $[C]_{shoot}$ than those grown under $a[CO_2]$. $[C]_{shoot}$ was lower in the drought-stressed plants as compared with that of the well-watered. Shoot N concentration ($[N]_{shoot}$) was significantly affected by both CO_2 and water treatment (Fig. 5b). $[N]_{shoot}$ was 20.8% lower in $e[CO_2]$ than in $a[CO_2]$ and was 48.6% higher in the drought-stressed than in the well-watered plants, respectively. The ratio of C to N in shoot ($[C:N]_{shoot}$) was markedly affected by CO_2 , water and $CO_2 \times$ water interaction (Fig. 5c). The $[C:N]_{shoot}$ of plants grown under $e[CO_2]$ was 27.7% higher than that grown under $a[CO_2]$. For the drought-stressed plants, $[C:N]_{shoot}$ was 32.8% lower compared with the well-watered controls (Fig. 5c).

3.6. Concentration of ABA in leaf and xylem sap and relation with g_s

The ABA concentration in leaf ($[ABA]_{leaf}$) and xylem sap ($[ABA]_{xylem}$) increased exponentially with the depletion of soil water for plants grown under both CO_2 environments (Fig. 6a–b). The relationship of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ to g_s under $a[CO_2]$ and $e[CO_2]$ were presented in Fig. 7a–b. g_s decreased linearly with the increase of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ under both CO_2 environments, while no significant difference in the slope of the regression lines between $a[CO_2]$ and $e[CO_2]$ was observed; however, the intercepts of the regression lines were significantly differed between the two CO_2 environments.

4. Discussion

Under non-drought stress conditions, the g_s in plants grown at $e[CO_2]$ was significantly lower compared to those grown at $a[CO_2]$ (Fig. 3a), in accordance with the early findings that $e[CO_2]$ induced stomatal closure thus lowering g_s (Kang et al., 2002; Azoulay-Shemer et al., 2015; Easlon et al., 2015). The signals inducing stomatal closure under $e[CO_2]$ are still elusive, and both ABA-dependent and independent pathway could exist (Hus et al., 2018). Early study by Raschke (1975) illustrated that ABA could enhance the stomatal CO_2 response under sufficient soil moisture. Additionally, Tazoe and Santrucek (2015) reported that ABA levels might mediate the stomatal closure induced by $e[CO_2]$. Recent study by Hus et al. (2018) suggested that $e[CO_2]$ triggers stomatal closure through an ABA-independent pathway downstream of OST1/SnRK2 kinases. In the present study, plants grown under both $a[CO_2]$ and $e[CO_2]$ had similar level of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ at onset of the drought stress (Fig. 6). This result indicates $[ABA]$ in leaf or xylem might have not played an essential role in modulating stomatal aperture in tomato plants in response to $e[CO_2]$, consistent with the findings of Yan et al. (2017). However, it should be noted that as the transpiration rates of the plants differed significantly between the $a[CO_2]$ and $e[CO_2]$ treatments, the ABA delivery rates could be affected even though the $[ABA]_{xylem}$ was identical between the two CO_2 environments, which may modulate the ABA signalling (Yong et al., 2000). The decrease of g_s in plants grown

Table 2
Leaf relative water content (RWC), leaf water potential (ψ_l), osmotic potential (ψ_{π}) and turgor press (ψ_p), water consumption (WU), dry matter increase (ΔDM) and water use efficiency (WUE), leaf area (LA) and special leaf area (SLA) of well-watered (W) and drought-stressed (D) plants under 400 ppm ($a[CO_2]$) and 800 ppm ($e[CO_2]$) environments in the final harvest. The data in the table was mean \pm standard error of the means (S.E.) (N = 3–4).

	RWC	ψ_l (MPa)	ψ_{π} (MPa)	ψ_p (MPa)	WU (cm^3)	ΔDM (g)	WUE ($kg \cdot m^{-3}$)	LA (cm^2)	SLA ($cm^2 \cdot g^{-1}$)
400 ppm									
Well-watered	0.87 \pm 0.05	-0.45 \pm 0.03	-0.72 \pm 0.08	0.27 \pm 0.10	1812.25 \pm 41.74	11.86 \pm 0.49	6.54 \pm 0.14	2598.05 \pm 64.16 a	18170 \pm 5.49
Drought	0.68 \pm 0.03	-1.12 \pm 0.02	-1.32 \pm 0.08	0.20 \pm 0.10	1178.83 \pm 17.96	6.01 \pm 0.33	5.10 \pm 0.33	1514.11 \pm 65.74 c	165.72 \pm 7.41
800 ppm									
Well-watered	0.87 \pm 0.02	-0.39 \pm 0.01	-0.76 \pm 0.07	0.37 \pm 0.07	1758.38 \pm 57.80	13.60 \pm 1.04	7.74 \pm 0.56	2593.19 \pm 45.03 a	156.89 \pm 8.64
Drought	0.68 \pm 0.02	-1.01 \pm 0.17	-1.28 \pm 0.10	0.27 \pm 0.19	1126.67 \pm 56.98	7.71 \pm 0.88	6.85 \pm 0.68	1757.65 \pm 66.90 b	156.50 \pm 2.18
P value from two-way ANOVA									
$[CO_2]$	0.90	0.09	0.98	0.24	0.07	0.01	< 0.01	0.01	0.01
$[Water]$	< 0.01	< 0.01	< 0.01	0.20	< 0.01	< 0.01	0.01	< 0.01	0.04
$[CO_2] \times [Water]$	0.83	0.56	0.40	0.84	0.97	0.96	0.30	0.01	0.05

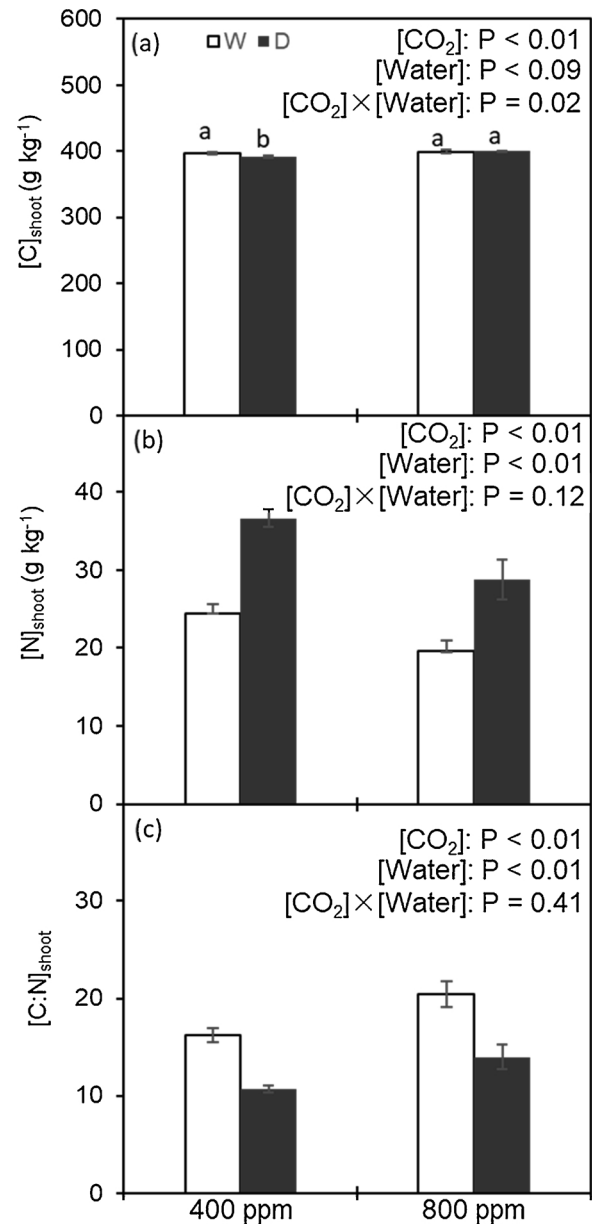


Fig. 5. Concentration of carbon, nitrogen and C/N ratio in shoot ($[C]_{shoot}$, $[N]_{shoot}$ and $[C:N]_{shoot}$) of well-watered (W) and drought-stressed (D) plants under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations in the final harvest. Error bars indicate the standard error of the means (S.E.) (N = 3–4). P value indicates the significant difference by statistical comparisons among the treatments.

under $e[CO_2]$ may be related to the lowered leaf [N] (data not shown) as Franks et al. (2009) found a positive correlation between g_s and leaf [N].

In the present study, both A_n and g_s become less sensitive to soil drying in plants grown under $e[CO_2]$ than under $a[CO_2]$ (Fig. 3a). Similar results have been reported by Yan et al. (2017) in tomato plants. These retarded responses of A_n and g_s to soil drying under $e[CO_2]$ could have significant implications in altering plant drought adaption and WUE (Fig. 3b). The reduced sensitivity of g_s to soil drying plants at $e[CO_2]$ could be due to an impaired stomatal response to root-to-shoot ABA signaling (Yan et al., 2017). While the greater vapor pressure deficit (VPD, Fig. 1d) in the $e[CO_2]$ greenhouse cell could have also modulated the g_s response to soil drying (Ray et al., 2000), though the effect of VPD on the FTSW threshold was variable among different studies. The delayed reduction of A_n and g_s during soil drying might

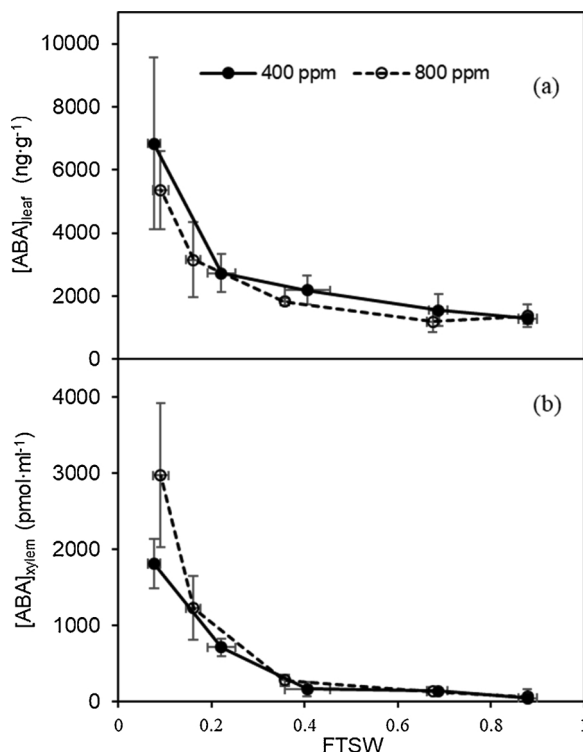


Fig. 6. Change of ABA concentration in tomato leaf ($[ABA]_{leaf}$) and xylem sap ($[ABA]_{xylem}$) grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during progressive soil drying. Error bars indicate standard error of the means (S.E.) (N = 3–4).

enable the plants to accumulate more biomass, this would be a disadvantage under terminal drought which may potentially hasten mortality during severe drought (Will et al., 2013). On the contrary, plants grown at $a[CO_2]$ would conserve water by closing stomata at a higher FTSW threshold, and therefore might be a privilege under situation of prolonged drought.

It was well documented that ABA acts as an earlier chemical signaling before hydraulic signaling in the regulation of g_s to drought stress (Zhang and Davies, 1989; Zhang et al., 2018). Under moderate soil drying, g_s decrease linearly with the increase of $[ABA]_{leaf}$ and $[ABA]_{xylem}$ in both CO_2 environments (Fig. 7), indicating both root-borne ABA and leaf ABA could be involved in the regulation of stomatal

aperture. However, one should note in addition to ABA, other phytohormones, such as cytokinins, are also involved in stomatal regulation during drought and CO_2 elevation (Mansfield et al., 1990; Yong et al., 2000). Additionally, the slopes of the regression lines for plants grown under the two CO_2 conditions were identical implying that the responsiveness of g_s to ABA was unaffected by CO_2 . Nonetheless, the intercept of the regression lines under $e[CO_2]$ was significantly lower than those under $a[CO_2]$ (Fig. 7), affirming that $e[CO_2]$ induced decreased g_s could be ABA-independent.

Literatures has reported that $e[CO_2]$ could improve plant water relation due to the decrease of leaf g_s thereby T_r (Wullschlegel et al., 2002), though the response varied among plant species (i.e. Bunce, 1995). Here, no significant difference in RWC, ψ_i , ψ_π and ψ_p were observed of plant grown under $a[CO_2]$ versus $e[CO_2]$ (Table 2) even though g_s and T_r were both lowered in plants exposed to $e[CO_2]$ (Fig. 3). The reason could be related to the relatively higher VPD at $e[CO_2]$ environment (Fig. 1d), as Jurado et al. (2009) found that at ψ_i decreased at high VPD.

In this study, $[N]_{shoot}$ was significantly decreased both in the well-watered and the drought-stressed plants grown under $e[CO_2]$ as compared to those grown at $a[CO_2]$ (i.e., 20.0% and 21.3%, respectively); and the drought-stressed plants possessed higher $[N]_{shoot}$ than the well-watered plants under both $a[CO_2]$ and $e[CO_2]$ (Fig. 5b). $e[CO_2]$ increased plant biomass by 15.2% and 22.0% for well-watered and drought-stressed plants, respectively, which would lead to $[N]_{shoot}$ dilution ca. 13.2% and 18.0%, according to the diluted effect (Loladze, 2002). Therefore, the reduction of $[N]_{shoot}$ was not due solely to the dilution effect and a lower N absorption due to decreased transpiration mass flow (Fig. 3c) could also be involved (Taub and Wang, 2008; Myers et al., 2014). Shoot C concentration ($[C]_{shoot}$) in drought plant at $e[CO_2]$ was enhanced (Fig. 5a) leading to higher C:N in shoot ($[C:N]_{shoot}$, Fig. 5c), which indicated higher N use efficiency (NUE) was obtained in plants grown under $e[CO_2]$ (Reddy et al., 2010).

5. Conclusion

Conclusively, plants grown under $e[CO_2]$ delayed A_n and g_s response to progressive soil drying. The mechanism by which decreases of g_s in plants under $a[CO_2]$ could be regulated by both $[ABA]_{leaf}$ and $[ABA]_{xylem}$, while under $e[CO_2]$ at moderate drought could be ABA-independent. $e[CO_2]$ did not influence plant water relations in the well-watered plants and drought plants which most probably due to higher VPD under $e[CO_2]$. Additionally, $e[CO_2]$ decreased $[N]_{shoot}$ and enhanced $[C:N]_{leaf}$ both in well-watered plants and drought plants,

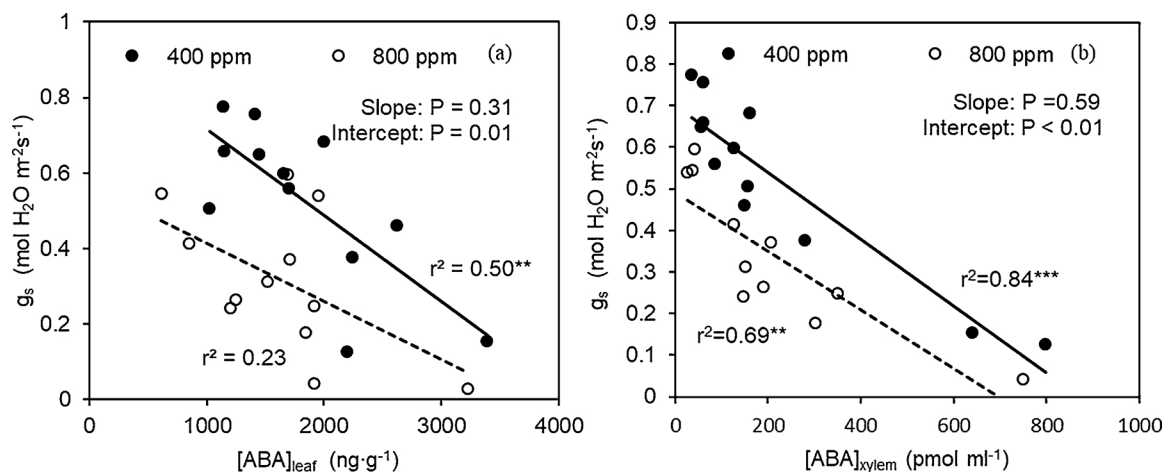


Fig. 7. Relations between leaf and xylem ABA concentration ($[ABA]_{leaf}$ and $[ABA]_{xylem}$) with stomatal conductance (g_s) of tomato plants grown under ambient (400 ppm) and elevated (800 ppm) atmospheric CO_2 concentrations during moderate progressive soil drying. ** indicates the regression lines were statistically significant at $P < 0.01$ level. P value indicates the significant difference in slopes and intercepts between the two regression lines ($P < 0.05$).

indicating $e[\text{CO}_2]$ could improve nitrogen use efficiency (NUE).

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